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Hsr-omega, A Novel Gene Encoded by a *Drosophila* Heat Shock Puff*

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Abstract. Although originally identified because of its abundant transcription in heat shock, the *hsr-omega* gene is active, at generally lower levels, in non-stressed cells. The locus produces an unusual set of three transcripts. Evidence from a variety of experiments suggests that one of these transcripts acts in the nucleus, possibly to regulate the activity of a nuclear protein. Another of the transcripts appears to act in the cytoplasm, possibly monitoring or regulating some aspect of translation. The two transcripts together could have a role in coordinating nuclear and cytoplasmic activity. A number of processes occur in eukaryotic cells in which nuclear and cytoplasmic activities need to be coordinated; we suggest that *hsr-omega* plays a role in such coordination.

Introduction

Biologists have often found that a biological peculiarity in one organism can be exploited to study general questions that apply to many organisms. The polytene chromosomes that are found in some cells in *Drosophila* and some other organisms are examples of such a biological peculiarity. Studies of these chromosomes have contributed significantly to our understanding of how gene activity changes during development or in response to various agents (see Beermann, 1972; Hennig, 1987).

Polytene chromosomes are giant chromosomes, made up of many chromatids lying side by side in precise alignment. Although polytene chromosomes are condensed enough to allow cytological mapping, they are interphase chromosomes and thus allow us to actually see, *in situ,* chromatin structures involved in transcription and DNA replication. When a gene is being very actively transcribed, the site of that gene frequently undergoes a localized puffing of the many DNA strands that make up the chromosomes. Many transcribed regions do not make detectable puffs, but when a puff is seen, it is always a sign of very active transcription. A new puff indicates that transcription at the puff site has either been turned on, or turned up very sharply (Bonner and Pardue, 1977).

About 25 years ago, F. Ritossa (1962, 1964) found that when *Drosophila* larvae were placed at 37°C for a short period, nine new puffs were induced, suggesting that this heat shock induced nine new genes (Fig. 1). The genes were scattered over the chromosomes but still seemed to be controlled coordinately. Further, the same set of genes could be induced by other kinds of stresses, including a wide variety of chemicals. This induction was reversible; the puffs regressed as soon as the stress was removed. This was clearly a fascinating set of genes, but a good many years were to pass before it became technically possible to find out what the genes coded for. When that happened, the puffs were found to encode a small set of proteins now called the heat shock proteins, or hsps (see Ashburner and Bonner, 1979). The name "heat shock" is really a historical one because the proteins were first identified after a 37°C heat shock. We know now that these proteins are also induced by a variety of stresses and they are sometimes called the stress proteins. More recently it has become apparent that all of the hsps also have roles in non-stressed cells (for recent reviews on heat shock see Lindquist, 1986; Lindquist and Craig, 1988; Pardue *et al.,* 1988). In some cases, the hsps in non-stressed cells are encoded by the same genes that

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show increased activity in response to heat shock. In other cases, the hsps may be encoded by closely related genes, some activated by heat shock and some regulated in other ways.

After hsps had been identified in *Drosophila*, it became evident that all organisms make very similar sets of stress proteins. Animals, plants, and bacteria all show this heat shock response, so presumably the response has been around almost as long as cells have. The conservation of the major hsps is striking. For example, the hsp70 of *Drosophila* has 48% amino acid identity with the equivalent hsp of *E. coli* (Bardwell and Craig, 1984). Although the major proteins produced in the heat shock response are strongly conserved, the stimuli that induce the response vary from organism to organism and reflect the conditions under which the organism lives. For instance, the temperatures that induce heat shock in *Drosophila* cells are well below those that heat shock mammalian cells. This is not surprising because *Drosophila* cells usually live 10–20 degrees below the temperature of mammalian cells.

Evolutionary conservation argues that the heat shock response is very important, yet we know only a little about how it helps the organism. Clearly the response helps cells endure, for a short time, temperatures slightly above what they normally tolerate. If cells are subjected to a mild heat shock and make a low level of heat shock proteins and RNA, then they can survive temperatures that would kill them if they were moved directly to those temperatures.

We do not know how any of the hsps protect the cell from the heat shock, but studies of these proteins in nonstressed cells are showing that the hsps have very interesting roles in normal cells in addition to their roles in stressed cells. Three major families of hsps are now known to be conserved in plants, animals, and bacteria. For each of these families there is evidence suggesting that members act as "molecular chaperones." That is, these proteins appear to regulate the association of proteins with other macromolecules. The hsp70 family has many members; one of them has been implicated in translocation of secretory proteins into the endoplasmic reticulum and another appears to translocate proteins into mitochondria (Chirico *et al.*, 1988; Deshaies *et al.*, 1988). The hsp 90 family (which includes the *Drosophila* hsp 82) appears to chaperone steroid hormone receptors and has also been found in association with some protein kinases (Catelli *et al.*, 1985). The bacterial hsp 70 is *dna K*, which is involved in protein-protein interactions in DNA replication (Georgopoulos *et al.*, 1989). A major bacterial hsp is *groEL*, which participates in bacteriophage assembly, although it probably has other roles (Herendeen *et al.*, 1979). Recently *groEL* has been shown to be related to an animal mitochondrial protein

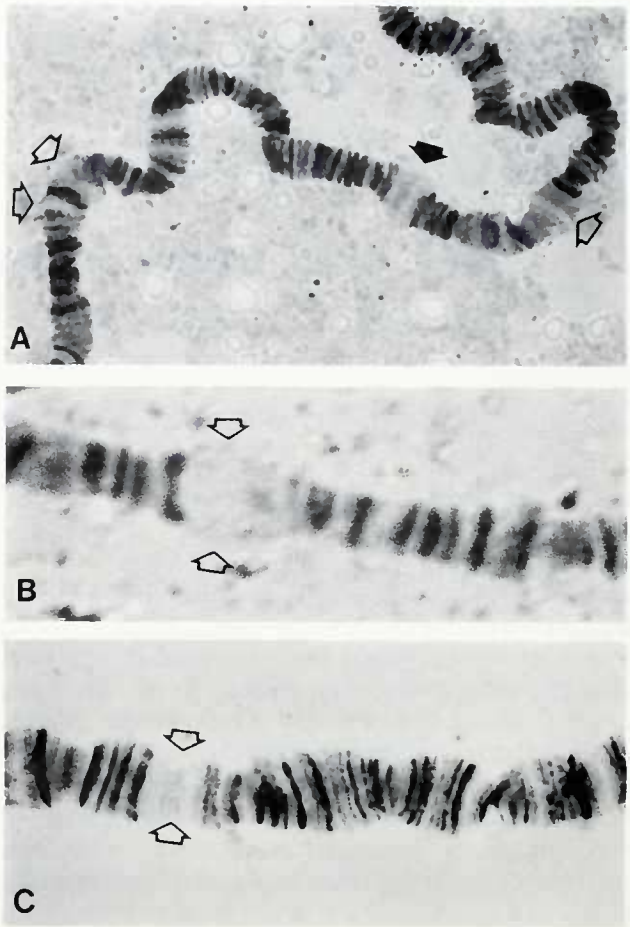

Figure 1. *Drosophila melanogaster* salivary gland chromosomes showing heat shock-induced puffing. (A) Part of chromosome 3 showing four of the major heat shock puffs (arrows, solid arrow indicates the 93D puff). × 900. (B) Higher magnification view of a 93D heat shock puff (arrows). ×1100. (C) The 93D region from a non-heat shocked larva. The region that puffs in heat shock is indicated by arrows. ×1100. The chromosomes in A and B are from larvae that had been heat shocked at 36°C for 30 min.

that is increased on heat shock (McMullin and Hallberg, 1988) and to a chloroplast protein that is involved in assembling the oligomeric enzyme Rubisco (Hemmingsen *et al.*, 1988).

Thus the polytene puffs have revealed the existence of perhaps the most basic cellular response to stress and led to extensive study of this homeostatic mechanism. In turn, the studies of heat shock have accelerated our understanding of a new class of proteins, the chaperonins. The polytene puffs have still more to tell us. For instance, all of the known *Drosophila* heat shock protein genes have been found. As expected, the genes are in the heat shock puffs, but there are still a few heat shock puffs with no known function. One that has intrigued us especially is in region 93D in *D. melanogaster*.

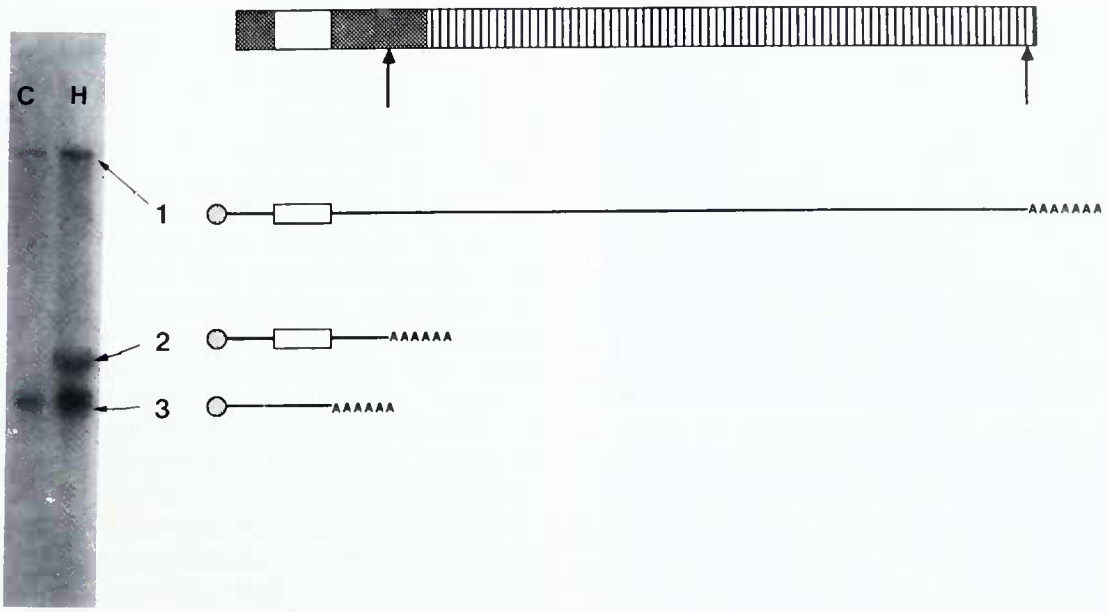

Figure 2. The transcripts of *hsr-omega*. The autoradiogram shows total RNA from control (lane C) and heat shocked (lane H) cultured *Drosophila* cells. The RNA has been gel fractionated, transferred to a filter, and hybridized with ^{32}P-labeled probe containing the sequence of transcript *omega 3* (and therefore complementary to a portion of the sequences of *omega 1* and *omega 2*, also). Longer exposures of the autoradiogram show that all three transcripts are present in the control cells (Garbe *et al.*, 1896). The top diagram shows the transcribed region of the gene. The shaded areas indicate the "unique portion" of the gene, much of which is incorporated in the cytoplasmic *omega 3* transcript. The white area represents the intron that is spliced out in the processing of *omega 3*. The striped area represents the region of small tandem direct repeats. The two arrows mark the polyadenylation signals used in the processing of *omega 2* (left arrow) and *omega 1* (right arrow). The three transcripts are diagrammed below the transcription unit.

The Unusual Puff at 93D

The puff at 93D is one of the very largest puffs, suggesting that it is actively transcribed during heat shock. Cytological studies have shown that 93D is unusual in a number of ways. (1) 93D is a bona fide member of the heat shock set, but it can also be induced by a number of agents that do not induce the other members of the family. (2) 93D contains large RNP granules never seen in other puffs. (3) 93D binds antibodies to several nuclear antigens not found in the other heat shock puffs. Every species of *Drosophila* studied has one (and only one) heat shock puff that has all these strange features (see Lakhotia, 1987).

The cytological studies strongly suggested that the unusual member of the heat shock puff set in each *Drosophila* species is homologous to the unusual member in each of the other species. We now have evidence that this is true and have named this puff the *hsr-omega* locus. (The name reflects its original identification as the locus encoding *h*eat *s*hock *R*NA *omega*, although we now think that the locus is important in almost all cells, whether or not they are heat-shocked.)

Although the homology story turned out happily in the end, it seemed for awhile that there was no sequence homology between these puffs. Berendes and his colleagues in The Netherlands isolated a cDNA clone from the *D. hydei* 2-48B puff (*i.e.*, the *D. hydei* heat shock puff with unusual features). The cDNA consisted of tandem 115 nt repeats (Peters *et al.*, 1984). The clone was used to select a cosmid clone of *D. hydei* DNA that covered the entire puff region. Neither the cosmid nor the cDNA showed any cross-hybridization with *D. melanogaster* DNA. Thus the 2-48B gene appeared to have no homologue in *D. melanogaster* DNA. However, we now know that 2-48B is the *D. hydei* homolog of *D. melanogaster* 93D (Garbe *et al.*, 1986). Apparently the sequence at this locus has evolved faster than the sequences of the other heat shock genes, whereas the phenotype, as deduced from cytological puffs, has been conserved. As discussed below, our studies of the 93D gene substantiate, and help to explain, this somewhat paradoxical conclusion.

We began our study of the 93D puff by using a cloned gene to determine the structure of the locus and its transcripts. The structure is quite different from that of the other heat shock genes or any other known gene (Fig. 2).

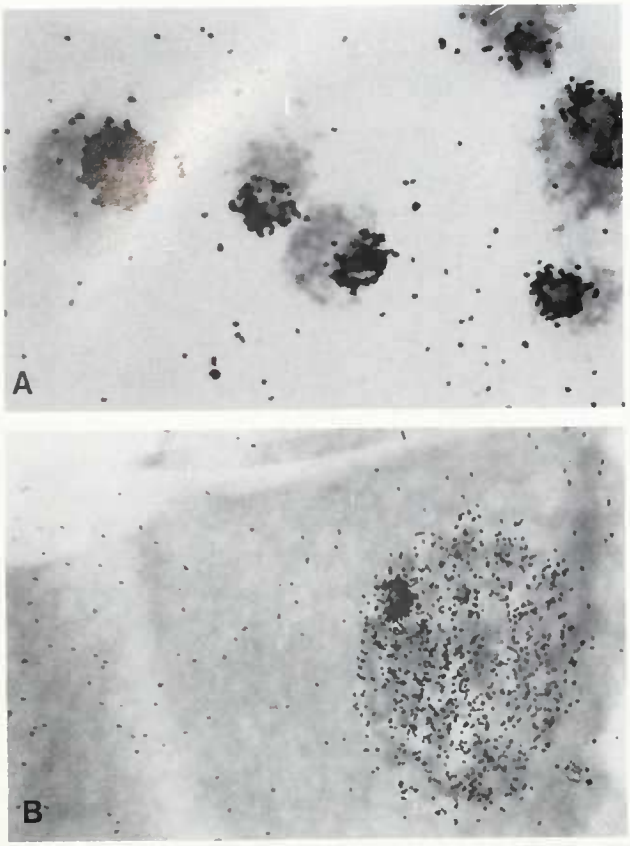

Figure 3. Autoradiograms showing the localization of *omega 1* to the nucleus in both diploid cells (A) and polytene cells (B). In both experiments the cells were fixed, permeablized, and hybridized with a ^{3}H-labeled probe complementary to the *hsr-omega* repeats (Bendena *et al.*, 1989a). The cells in (A) are cultured cells. ×1100. (B) shows the nucleus and part of the cytoplasm from a larval salivary gland cell. ×700. The cells in (A) have been heat shocked for 1 h at 36°C. The cell in (B) has not been heat shocked. Heat shock increases the amount of *omega 1* in the cell but does not affect the nuclear location.

The transcribed region is greater than 10 kb and can be divided into two parts: a 5′ unique region (approximately 3 kb) and a region of tandem repeats (8–25 kb, depending on the allele). These repeats are found nowhere else in the genome. Typical heat shock transcription signals can be found upstream from the start of transcription. Because the transcript is regulated in multiple ways, there must be other control signals also, but these have not yet been dissected (Garbe *et al.*, 1989).

In both normal and stressed cells, the *hsr-omega* locus produces a distinctive set of three major transcripts, all starting from the same nucleotide (Fig. 2). The largest transcript, *omega 1*, contains all of the sequence between the start and the second termination site (which is marked by a poly-adenylation signal). *Omega 1* is not a precursor for the other transcripts. It remains in the nucleus, and its turnover is controlled differently from the other two transcripts (Fig. 3). Its most remarkable feature is the segment of over 8 kb of tandem repeats. Each repeat is only 284 bp long, and the repeats differ from each other by less than 10% (Garbe *et al.*, 1986). The second 93D transcript, *omega 2*, appears to be made by an alternative termination near the first polyadenylation signal. *Omega 2* is also nuclear but it seems to be a more-or-less typical precursor that is spliced to make the cytoplasmic transcript, *omega 3*. These three transcripts have typical RNA processing sequences for splicing, polyadenylation, etc., but show no other similarities to known RNAs that might provide clues to function (Garbe *et al.*, 1989). We have, therefore, used a number of techniques—biochemical, cytological, and genetic (both classical and reversed)—to search for function (Bendena *et al.*, 1989). The results of these experiments will be discussed below.

Our studies to date suggest a working hypothesis about the function of the *hsr-omega* locus. Although not the only possible hypothesis, this seems to provide the simplest explaination of all the observations. Briefly, we suggest that *omega 1* serves to bind some component in the nucleus, thereby affecting either the activity or the level of this component in the nucleus. Because levels of *omega 1* vary quickly in response to cellular conditions, this binding would make the activity or the nuclear level of the bound component respond rapidly to those conditions and could thus serve a regulatory role. In the case of the cytoplasmic transcript, *omega 3*, we propose that the RNA, not a protein product, is important. One possibility is that the level of *omega 3* RNA reflects the rate of protein synthesis at any given time and provides a way to link another cell process to this rate. Finally, we wonder why both the nuclear and the cytoplasmic transcripts of *hsr-omega* come from the same start site. Does this in some way coordinate the initial levels of the nuclear and cytoplasmic transcript? A number of processes in eukaryotic cells have nuclear and cytoplasmic activities that must be coordinated. Perhaps this gene plays a role in such coordination.

Evolutionary Comparisons of *Hsr-omega* Genes

When we found that 93D had short tandem repeats, we were struck by its resemblance to the *D. hydei* 2-48B gene. Although the *D. melanogaster* repeats (284 bp) are larger than those in *D. hydei* (115 bp), such long stretches of repeats in a transcribed region were too unusual to dismiss as unrelated, notwithstanding the previous evidence that the genes shared no sequence homology. We used small pieces of the *D. melanogaster* DNA sequence to probe DNA from the cosmid clone of the *D. hydei* 2-48B locus. Our hybridization results confirmed those of Berendes and his colleagues, with one significant exception (Garbe and Pardue, 1986). Most fragments showed no cross-hybridization at any stringency. The small re-

gion that did show cross-hybridization, however, encouraged us to clone and sequence the rest of the *D. hydei* gene. The sequence analysis showed that the cross-hybridization was due to 60 nt of perfect homology and, surprisingly, 40 of the conserved nucleotides were in the intron, while the rest extended beyond the 3′ splice site. In spite of the differences in sequence, the *D. hydei* gene had the same structure as the *D. melanogaster* gene, and the location of the conserved region was the same in both genes. The same conserved sequence has now been found in the *hsr-omega* loci of all of the other *Drosophila* species that have been studied. The homology can be detected by *in situ* hybridization to polytene chromosomes. The sequence homology has been used to clone the *hsr-omega* gene from *D. pseudoobscura*. The three cloned genes enable us to compare *hsr-omega* sequences from distantly related *Drosophila*: *D. melanogaster* and *D. pseudoobscura* are separated by 46 million years, and both are separated from *D. hydei* by 60 million years (Beverley and Wilson, 1984).

Our studies of the *D. hydei* and *D. pseudoobscura* genes have shown that the structure of the *hsr-omega* locus is conserved, although the sequences have diverged (Garbe *et al.*, 1988). In each *Drosophila* species, the locus has a unique region followed by a long string of tandem repeats. In each species, there are three transcripts of approximately the sizes found in *D. melanogaster*. In each species the 60 nt conserved region is in the intron and overlaps the 3′ splice site.

In spite of the strong evolutionary divergence, the sequence comparisons give useful clues about possible functions. The clues are strong because the evidence that so much of the sequence can change increases the significance of the parts that have not changed.

The unique portion of the gene

In any pairwise alignment of the sequences from *D. melanogaster*, *D. hydei*, or *D. pseudoobscura*, the longest conserved sequence is the 60 nt around the 3′ splice site (Garbe *et al.*, 1988). (The conserved region rises to 62 nt when the *D. melanogaster* sequence is compared with that of *D. pseudoobscura*.) This conserved sequence might be necessary for splicing in heat shock; however that seems unlikely if one considers a similar sequence comparison of the *hsp83* gene. The hsp83 transcript is also spliced in heat shock and it shows very little sequence conservation in the intron (Blackman and Meselson, 1986). The hsp83 exon shows significant sequence conservation, but because the exon codes for protein, it is probably the protein sequence that is conserved, an explanation that does not hold for the 93D exon, which does not encode a protein. At this point, the reason for this conserved sequence is a puzzle.

With the exception of the 60–62 bp conserved region, pairwise alignments of the rest of the unique region of *hsr-omega* show few stretches of conserved sequences longer than 10–20 bp. Interestingly, some of the longest regions of homology surround sites that our RNA studies indicate to be important for RNA processing and function. These include 14–16 bp (depending on the species compared) at the 5′ splice site, and 15–21 bp at the polyadenylation signal. In addition, the transcription start site shows conservation between the species and also has five of the six specific nucleotides that are conserved in all *Drosophila* heat shock mRNAs, except the hsp83 mRNA. The sequence alignments also show why there is so little cross-hybridization between *hsr-omega* genes. There have been many short insertions and deletions that eliminate runs of sequence long enough to hold a hybrid. The deletions and insertions tend to balance out, so the sizes of the exons and introns are conserved.

Another conserved feature of the *hsr-omega* genes is the lack of long open reading frames (Garbe *et al.*, 1986). The only open reading frames (ORFs) are very short (shorter than those found on the opposite, non-transcribed strand). A comparison of the transcripts from the three *Drosophila* species shows only one ORF that is at all conserved in location or sequence (Garbe *et al.*, 1989). The location is interesting because, in each species, this ORF is the first one that is in a sequence context thought to be favorable for translation. The sequence conservation is not very strong; only the first four amino acids, plus a few other scattered amino acids, would be the same in all three translation products. Even the size of the ORF varies; the translation product would contain 23, 24, and 27 amino acids, depending on the species. In spite of the low level of conservation, other studies (discussed below) strongly suggest that this ORF is important in the function of the *omega 3* transcript.

The general conclusion from these sequence studies on the unique part of the gene seems to be that the cell is conserving the ability to make, splice, and polyadenylate an RNA of this size. This conclusion is based on the small conserved regions, the significance of which we already know. There are other small conserved segments that we cannot now decode; these regions probably also have functional importance.

The tandemly repeated segment of the gene

Restriction enzyme mapping of DNA from *hsr-omega* loci indicates that, in all *Drosophila* species, this locus has >8 kb of short tandem repeats. Repeats from both *D. melanogaster* and *D. hydei* have been sequenced. Within each *Drosophila* species the repeats show <10% divergence, but between species the repeats differ in both size and sequence. There is, however, a conserved 9 nt seg-

ment, AUAGGUAGG, that is found once in the 115 nt repeat of *D. hydei* and twice in the 284 nt repeat of *D. melanogaster* (Garbe *et al.*, 1986). The 9 nt segment thus occurs at about the same frequency along the transcripts. *D. pseudoobscura* repeats have not yet been sequenced, but preliminary evidence suggests that they will also have the 9 nt sequence. The sequence, AUAGGUAGG, is in the size range of sequences that have been shown to serve as binding sites for proteins and RNAs. The sequence may serve as a binding site in the *omega 1* RNA. If so, the repeats may be a device to maintain a certain number and spacing of copies of the binding site.

The evolution of the *hsr-omega* repeats appears much like that of satellite DNA. That is, the repeats are very homogeneous within each species, but diverge rapidly between species. The *D. melanogaster* repeat will hybridize only with DNA from the sibling species, *D. simulans*, and, even in this case hybrid stability is reduced, and restriction site differences indicate some sequence change.

The Cytoplasmic Transcript, *Omega 3*

The cytoplasmic RNA, *omega 3*, is spliced and polyadenylated, two characteristics usually associated with mRNAs, yet the only open reading frames (ORFs) are small and show very little conservation. Surprisingly, *omega 3* is found on polysomes (monosomes and disomes) by all known criteria (Fini *et al.*, 1989). The localization in control cells does not change when the cells are heat shocked. In both cases almost all of the transcript was loaded on the polysomes; rarely is any of the transcript free. In control cells, *omega 3* turns over rapidly but is stabilized by all inhibitors of protein synthesis (Fig. 4) (Bendena *et al.*, 1989). There is now evidence that turnover of certain mRNAs is linked to their presence on polysomes (Hunt, 1988). Although the studies described below suggest that *omega 3* is not an mRNA, *omega 3* shows a specific and rapid turnover when it is associated with active polysomes, as do these mRNAs (Bendena *et al.*, 1989b).

These evidences of an association between *omega 3* and protein synthesis have led us to search very hard for an *hsr-omega* translation product (Fini *et al.*, 1989). That search has been unsuccessful; but we have obtained indirect evidence that the small, partially conserved, ORF in *omega 3* is translated. The indirect evidence comes from experiments with recombinant DNA molecules in which a bacterial chloramphenicol acetyltransferase (CAT) gene was joined to the 5′ part of the *hsr-omega* sequence (Fig. 5). The constructs were stably transformed into *Drosophila* cultured cells and tested for their ability to direct synthesis of mRNA and CAT protein. The experiments showed that *omega 3* ORF blocks translation of a CAT gene placed just 3′ to it, as long as

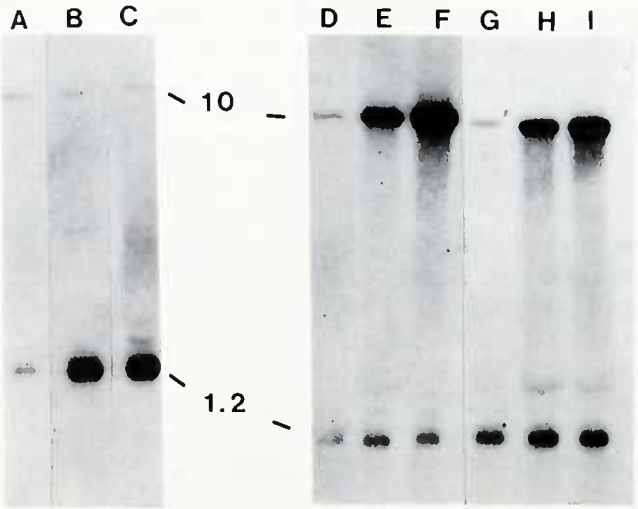

Figure 4. Autoradiograms showing differential regulation of the nuclear and cytoplasmic transcripts of *hsr-omega*. Inhibitors of protein synthesis lead to preferential accumulation of the cytoplasmic transcript, *omega 3*. Drugs that induce puffing of 93D without inducing other heat shock loci (*e.g.*, colchicine, benzamide) lead to preferential accumulation of the nuclear transcript, *omega 1*. To make the autoradiogram, RNA from cultured Drosophila cells has been fractionated and probed with the *omega 3* sequence, as in Figure 2. RNA from control cells (A, D, G), cells treated for 2 h with 10^{-4} *M* cycloheximide (B), cells treated for 2 h with 10^{-7} *M* pactamycin (C), cells treated with 10 m*M* benzamide for 12 h (E) or 24 h (F), cells treated with 100 μg/ml colchicine for 12 h (H) or 24 h (I). The 10 kb *omega 1* and the 1.2 kb *omega 3* transcripts are indicated.

the *omega 3* ORF has a termination codon so that the ribosome must reinitiate in order to translate CAT. In contrast, when the *omega 3* ORF is fused in frame to CAT, a CAT protein of appropriately larger size is produced. These transformation experiments give evidence that the *omega 3* ORF is translated *in vivo*, although no product can be detected. Thus the product of the ORF must either be degraded or be sequestered very rapidly. We think the first alternative is most likely because we have been unable to find any evidence of the product, even in the transformed cells where the excess product might be expected to saturate or slow a sequestration mechanism (Fini *et al.*, 1989).

Taken together, the studies on *omega 3* have suggested the following working hypothesis: the translation of the *omega 3* ORF functions to allow a polysome-associated turnover of *omega 3* and this turnover in some way serves to monitor, or regulate, some aspect of protein synthesis in the cell. Possibly, the *omega 3* RNA, or a degradation product of this RNA, has a function with a rate determined by the rate at which *omega 3* turns over on the polysomes. Admittedly this is highly speculative, but polysome-associated turnover may be common for RNAs that can be turned over rapidly (Hunt, 1988). The turnover of *omega 3* would reflect the level of protein

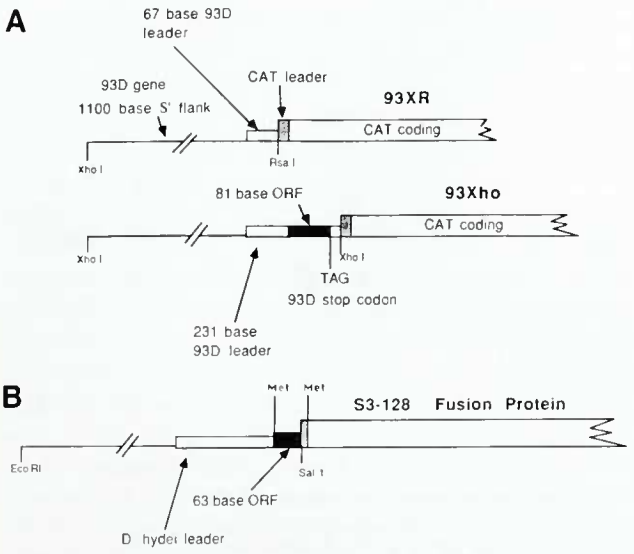

Figure 5. Recombinant DNA constructs used to analyze the translation of the most conserved small open reading frame (ORF) in *hsr-omega*. (A) The 93Xho construct was made by joining a bacterial chloramphenicol acetyltransferase (CAT) gene to DNA from the *D. melanogaster hsr-omega* gene. The junction was 30 nucleotides past the termination of the small ORF. The 93XR construct was made by deleting the small ORF from 93Xho. If the small ORF is translated *in vivo*, the ribosomes would not be expected to reinitiate on the CAT gene. Therefore the 93Xho construct would not be expected to direct the synthesis of CAT while the 93XR construct, where the CAT translation start is not blocked, should. When the constructs were stably transformed into *Drosophila* cultured cells, both constructs yielded abundant RNA but only the 93XR CAT gene was translated. (B) The S3-128 construct was made using the *D. hydei hsr-omega* gene. In this case the bacterial CAT gene was joined in frame to the conserved ORF so that ribosomes translating the ORF should continue translating into the CAT gene and yield CAT protein that is larger by the size of the ORF peptide. Cells carrying this construct do produce the predicted larger CAT. All of the experiments give strong evidence that the *hsr-omega* ORF is translated *in vivo* (Fini *et al.*, 1989).

synthesis at any particular time and could link some other cellular process to this level.

The Nuclear Transcript, *Omega 1*

One of the questions that we had about *hsr-omega* was whether all of the agents that induced the puff were acting on the *hsr-omega* sequences. Puffs usually involve more DNA than is actually transcribed, so a puff in the 93D region might indicate activation of a transcription unit that is not *hsr-omega*. The question had interesting implications. If the agents that induce the *hsr-omega* puff, but not the rest of the heat shock puffs, were inducing the transcripts that we are studying, it would suggest that *hsr-omega* is more sensitive to its environment than the other heat shock loci. Other experiments had shown that treatments inducing a puff at 93D blocked induction by a second agent, if the two inducers are applied in a relatively short time (Lakhotia, 1987). If all the inducers were acting on the *hsr-omega* gene, the observation that one inducer blocks activation by a second agent suggests that the *hsr-omega* locus can autoregulate, with products of the first induction inhibiting later induction. We have tested several inducers (Bendena *et al.*, 1989) and have found that all of the inducers do act on the *hsr-omega* locus (Fig. 4). These results suggest a responsiveness to external agents and an autoregulation that could characterize a regulatory locus. This sensitivity to the environment is consistent with the rapid response seen in heat shock in which the 93D region puffs slightly before the other loci and also returns to control levels more rapidly when cells are returned to normal temperature (Fig. 6).

These experiments with the other inducers gave an unexpected result. The level of *omega 1*, but not *omega 2* or *omega 3*, is increased by the agents that induce the 93D puff without inducing the rest of the heat shock loci (Fig. 4). The level rises rapidly in response to the agent, remains high so long as the agent is present, and drops rapidly when the agent is removed. Thus, the level of *omega 1* at any time reflects something that the cell perceives in its environment. The *omega 1* transcript accu-

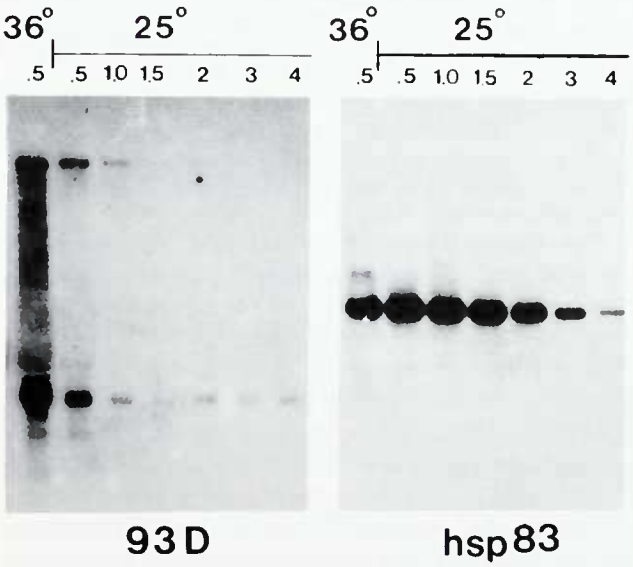

Figure 6. Autoradiogram showing that *hsr-omega* transcripts return to control levels more rapidly than the hsp83 transcripts upon recovery from heat shock. Cultured *Drosophila* cells were heat shocked for 30 min at 36°C and then returned to 25°C. Aliquots were taken at times indicated above each lane and the RNA was analyzed as in Figure 2. The panel marked 93D shows the *hsr-omega* transcripts as detected by the probe for the *omega 3* sequence. The panel marked hsp83 shows the same samples probed for the sequences encoding the D. melanogaster hsp83. The *hsr-omega* transcripts return to control levels at 1–1.5 h while the hsp83 transcripts do not return to control levels until 4 h after return to 25°C. (The autoradiogram shows only samples taken up to the time at which hsp83 returned to control levels.)

(er7	er4)	(er6)	(er5)	(er14)	(er2	e)	(er1)	(er8	er11	er9)	(er3)	(er19	er13	er16)
EC3	GC15	GC20	GC18	DC1	GC11		GC8	EC13	EC36	GC23		GC30	EC40	GC27
EC4	GC16	EC7	GC19		EC21		GC31	EC17	EC38	EC15			EC43	EC44
EC8	GC17	EC11	GC21		EC24		EC19	EC28						
EC9	EC2		EC1				EC20	EC32						
EC10	EC16		EC18				DC3	EC37						
EC30	EC25		EC26											
	EC27													
	EC33													

Figure 7. Diagram of the chromosomal deletions that bracket the *hsr-omega* locus and of the lethal complementation groups in that region in *D. melanogaster*. The lines indicate the relative locations and sizes of the three deletions. The brackets indicate the region of overlap of Df(3R)e^{Gp4} and Df(3R)GC14, which contains the *hsr-omega* locus. The complementation groups are listed below in order. In those cases where the order has not been established, the complementation groups are enclosed in parentheses. Below the name of each complementation group are given the names of the individual mutations that have been recovered in that group. The region of overlap of Df(3R)e^{Gp4} and Df(3R)GC14 is designated complementation group er3; it appears to contain only the *hsr-omega* locus but this has not been formally proven. Animals carrying both deficiencies and, therefore completely lacking er3, grow very poorly and do not survive to adulthood. Thus er3 appears to be an essential locus, but no point mutations in er3 have been recovered. Complementation group e is the *ebony* locus; several mutations in this group were recovered but were not scored. Because multiple mutants have been recovered in most of the complementation groups, it appears that er3 is unusually resistant to mutagenesis. Df(3R)ep1 removes the heat shock control elements, but not the constitutive activity of *hsr-omega*. Animals carrying this deletion and Df(3R)GC14 are fully viable at optimum temperatures but are much more sensitive to slightly increased temperatures than wild type flies.

mulates around the nucleus, apparently just under the nuclear membrane (Fig. 3).

The striking feature of the *omega 1* sequence is the array of tandem repeats containing an evolutionarily conserved potential binding site for a protein or RNA. The idea that the *hsr-omega* transcript binds protein is attractive because one of the distinctive features of the locus is the presence of 300 nm RNP granules not seen at any other locus. These granules could be formed by *omega 1* and its bound protein. This structural evidence, coupled with the evidence that levels of this RNA can change rapidly, suggests that *omega 1* might sequester variable amounts of a protein or RNA in response to variable cell conditions. The binding might affect the activity of the bound agent, either positively or negatively, or it might simply change the concentration that is present in the nucleus. In any case, such binding could allow *omega 1* to play a regulatory role for some aspect of cell metabolism. One way to test this hypothesis is to try to find a molecule that specifically binds to the *omega 1* RNA. We have recently found that an *in vitro* RNA transcript containing only the repeats from *omega 1* binds specifically to three bands on a blot of gel-fractionated *Drosophila* proteins: one band migrates at about 170 kD and a doublet migrates at 53–55 kD. The bands could represent a set of related proteins; alternatively, the smaller doublet could represent monomers or degradation products of the 170 kD band. These studies are preliminary, but the characterization of the proteins represented by these gel bands should give us useful information about the function of *omega 1*.

Genetic Analyses of *Hsr-omega*

Our genetic studies (Mohler and Pardue, 1984) are consistent with the hypothesis that *hsr-omega* has some sort of regulatory role. We have identified two chromosomal deletions, e^{Gp4} and GC14, that completely remove the *hsr-omega* sequences. e^{Gp4} removes 11 complementation groups proximal to *hsr-omega*, and GC14 removes 3 complementation groups distal to *hsr-omega*, but the 2 deficiencies appear to overlap only for *hsr-omega* (Fig. 7). Extensive attempts to recover point mutations in the region of overlap gave no lethal, visible, or temperature-sensitive mutations. What we know about the sequence conservation of *hsr-omega* suggests that it

would be almost impossible to inactivate it by a point mutation (or even by a tiny deletion), so we are not surprised to get no *hsr-omega* mutants. The genetic evidence provides a strong argument that no other genes (of conventional mutability) are located in the region of overlap. If e^{Gp4}/GC14 heterozygotes are indeed homozygous deficient only for *hsr-omega*, then their phenotype indicates that *hsr-omega* is important in non-stress situations. These e^{Gp4}/GC14 heterozygotes hatch as well as normal sibs (but mothers had one *hsr-omega* gene) but grow very slowly, dying at all stages of development. None survive as adults.

More recently we have recovered another deletion, ep1, that removes six complementation groups proximal to *hsr-omega* and also deletes the heat shock transcription signals, but not the constitutive transcription signals or the transcribed sequence of *hsr-omega* (Garbe, 1988). Heterozygous ep1/GC14 flies are viable and fertile at normal growth temperatures (25°C) but cannot grow at 31°C, a temperature at which wild type flies grow (but do not make sperm). The simplest explanation of the analysis of these two heterozygotes is that e^{Gp4}/GC14 flies die because they lack any *hsr-omega* gene, and the ep1/GC14 flies die at 31°C because their only *hsr-omega* gene has no heat shock control. But it is formally possible (considering the two results together) that the overlap of e^{Gp4} and GC14 also includes a lethal gene distal to *hsr-omega*, while the overlap of ep1 and GC14 includes a temperature-sensitive gene proximal to *hsr-omega*. (The other possibility, that either phenotype is simply due to the sum of the hemizygous loci, was excluded by testing animals carrying larger deficiencies for this region.) We have mapped the breakpoint of the ep1 deficiency and find that it is just downstream of the closest heat shock transcription signal of *hsr-omega*. The breakpoints of the other two deficiencies lie outside the region that we have cloned (about 5 kb 5′ and about 1 kb 3′). The ep1 deletion shows that there is no undiscovered lethal gene 5′ to *hsr-omega* that could cause death of the eGp4/GC14 heterozygotes. (If there were, ep1/GC14 flies would also die.) A highly repeated element just past the 3′ end of *hsr-omega* has made walking in that direction difficult, and we have not pursued it because breakpoint mapping cannot definitively reveal whether the phenotype we observe is due to the *hsr-omega* gene or to some other gene. The proof must be genetic. Our mutagenesis experiments were extensive, and they detected no mutable gene in this region. Thus, the genetic evidence argues that *hsr-omega* is the only gene in the overlap region; however, the definitive test must be rescue of the mutants by P-element transformation with the cloned gene. In the meantime, we suggest that, if the two transheterozygote phenotypes are due to *hsr-omega*, then they are consistent with a regulatory role for this locus. One possible type of regulatory role would be to link two processes in a way that increases metabolic efficiency. Mutants lacking the regulator would have the two processes running freely, at considerable expense of energy. Such mutants might grow slowly and have trouble making it past crucial developmental points or surviving in sub-optimal environments, as do the putative *hsr-omega* deletions. Because *hsr-omega* produces both nuclear and cytoplasmic transcripts, we speculate that the locus acts to link a nuclear with a cytoplasmic process.

Conclusion

We now have several kinds of information about the *hsr-omega* locus. It is clearly different from other genes, and some of those differences might explain why the locus has not attracted attention before. It seems to be relatively insensitive to mutagenesis and so would not be easily picked up in genetic studies. (Non-protein coding genes may be generally less sensitive to mutagenesis because, while any frame-shift or stop codon can destroy the function of an mRNA, RNAs with other functions are not so strongly polar as mRNAs; many small deletions or base changes in a non-coding RNA may make small local perturbations that do not affect function.)

Like the heat shock response, which was identified from polytene puffs, the *hsr-omega* locus may be found in many organisms. Because the sequence is evolving so rapidly, it may not be possible to use hybridization to detect the DNA in other genera, and the search for the gene in other organisms must wait until the function is better understood. Nevertheless, we think that this locus, like the loci encoding hsps, is common to many organisms.

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Literature Cited

Ashburner, M., and J. J. Bonner. 1979. The induction of gene activity in *Drosophila* by heat shock. *Cell* **17:** 241–254.

Bardwell, J. C. A., and E. A. Craig. 1984. Major heat shock gene of *Drosophila* and the *Escherichia coli* heat-inducible dnaK gene are homologous. *Proc. Nat. Acad. Sci. USA* **81:** 848–852.

Beermann, W., ed. 1972. *Developmental Studies of Giant Chromosomes. Results and Problems in Cell Differentiation. Vol. 4.* Springer-Verlag, New York. 277 pp.

Bendena, W. G., M. E. Fini, J. C. Garbe, G. M. Kidder, S. C. Lakhotia, and M. L. Pardue. 1989a. *hsr-omega*: a different sort of heat shock locus. Pp. 3–14 in *Stress-Induced Proteins. ICN-UCLA Symposia on Molecular and Cellular Biology. Vol. 96,* M. L. Pardue, J. R. Feramisco and S. Lindquist, eds. Alan R. Liss, Inc., New York.

Bendena, W. G., J. C. Garbe, K. L. Traverse, S. C. Lakhotia, and M. L. Pardue, 1989b. Multiple inducers of the *Drosophila* heat shock locus 93D (*hsr-omega*): inducer-specific patterns of the three transcripts. *J. Cell Biol.* **108:** 2017–2028.

Beverley, S. M., and A. C. Wilson. 1984. Molecular evolution in *Drosophila* and the higher Diptera. II. A time scale for fly evolution. *J. Mol. Evol.* **21:** 1–13.

Blackman, R. K., and M. Meselson. 1986. Interspecific nucleotide sequence comparisons used to identify regulatory and structural features of the *Drosophila hsp82* gene. *J. Mol. Biol.* **188:** 499–515.

Bonner, J. J., and M. L. Pardue. 1977. Ecdysone-stimulated RNA synthesis in salivary glands of *Drosophila melanogaster*: assay by *in situ* hybridization. *Cell* **12:** 219–225.

Catelli, M. G., N. Binart, I. Jung-Testas, J. M. Renoir, E. E. Baulieu, J. R. Feramisco, and W. J. Welch. 1985. The common 90-kd protein component of non-transformed "8S" steroid receptors is a heat shock protein. *EMBO J.* **4:** 3131–3135.

Chirico, W. J., M. G. Waters, and G. Blobel. 1988. 70k heat shock related proteins stimulate protein translocation into microsomes. *Nature* **332:** 805–810.

Deshaies, R. J., B. D. Koch, M. Werner-Washburne, E. A. Craig, and R. Sheckman. 1988. A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. *Nature* **332:** 800–805.

Fini, M. E., W. G. Bendena, and M. L. Pardue, 1989. Unusual behavior of the cytoplasmic transcript of *hsr-omega*; an abundant, stress-inducible RNA which is translated but yields no detectable protein product. *J. Cell Biol.* **108:** 2045–2057.

Garbe, J. C. 1988. Characterization of heat shock locus 93D of *Drosophila melanogaster*. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA.

Garbe, J. C., W. G. Bendena, M. Alfano, and M. L. Pardue. 1986. A *Drosophila* heat shock locus with a rapidly diverging sequence but a conserved structure. *J. Biol. Chem.* **261:** 16,889–16,894.

Garbe, J. C., W. G. Bendena, and M. L. Pardue. 1989. Sequence evolution of the *Drosophila* heat shock locus *hsr-omega*. I. The non-repeated portion of the gene. *Genetics* **122:** 403–415.

Garbe, J. C., and M. L. Pardue. 1986. Heat shock locus 93D of *Drosophila melanogaster*: a spliced RNA most strongly conserved in the intron sequence. *Proc. Nat. Acad. Sci. USA* **83:** 1812–1816.

Georgopoulos, C., K. Tilly, D. Ang, G. N. Chandrasekhar, O. Fayet, J. Spence, T. Ziegelhoffer, K. Liberek, and M. Zylicz. 1989. The role of the *Escherichia coli* heat shock proteins in bacteriophage lambda growth. Pp. 37–47 in *Stress-Induced Proteins. ICN-UCLA Symposia on Molecular and Cellular Biology. Vol. 96*, M. L. Pardue, J. R. Feramisco, and S. Lindquist, eds. Alan R. Liss, Inc., New York.

Hemmingsen, S. M., C. Woolford, S. M. van der Vies, K. Tilly, D. T. Dennis, C. P. Georgopoulos, R. W. Hendrix, and R. J. Ellis. 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature* **333:** 330–334.

Hennig, W., ed. 1987. *Structure and Function of Eukaryotic Chromosomes. Results and Problems in Cell Differentiation. Vol. 14.* Springer-Verlag, New York. 325 pp.

Herendeen, S. L., R. A. van Bogelen, and F. C. Neidhardt. 1979. Levels of major proteins of *Escherichia coli* during growth at different temperatures. *J. Bacteriol.* **139:** 185–194.

Hunt, T. 1988. Controlling mRNA life-span. *Nature* **334:** 567–568.

Lakhotia, S. C. 1987. The 93D heat shock locus in *Drosophila*: a review. *J. Genet.* **66:** 139–157.

Lindquist, S. 1986. The heat-shock response. *Ann. Rev. Biochem.* **55:** 1151–1191.

Lindquist, S., and E. A. Craig. 1988. The heat-shock proteins. *Ann. Rev. Genet.* **22:** 631–677.

McMullin, T. W., and R. Hallberg. 1988. A highly evolutionarily conserved mitochondrial protein is structurally related to the protein encoded by the *Escherichia coli groEL* gene. *Mol. Cell. Biol.* **8:** 371–380.

Mohler, J., and M. L. Pardue. 1984. Mutational analysis of the region surrounding the 93D heat shock locus of *Drosophila melanogaster*. *Genetics* **106:** 249–265.

Pardue, M. L., J. R. Feramisco, and S. Lindquist, eds. 1988. *Stress-Induced Proteins.* Alan R. Liss, New York. 294 pp.

Peters, F. P. A. M. N., N. Luhsen, U. Walldorf, R. J. M. Moormann, and B. Hovemann. 1984. The unusual structure of heat shock locus *2-48B* in *Drosophila hydei*. *Mol. Gen. Genet.* **197:** 392–398.

Ritossa, F. M. 1962. A new puffing pattern induced by heat shock and DNP in *Drosophila*. *Experientia* **18:** 571–573.

Ritossa, F. M. 1964. Experimental activation of specific puff loci in polytene chromosomes of *Drosophila*. *Exp. Cell Res.* **35:** 601–607.